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TECHNICAL MANUSCRIPT 580

EFFECT OF RECIPIENT CELL CONCENTRATION ON TRANSFECTION WITH BACTERIOPHAGE DNA

Darrel D. Gwinn
William D. Lawton

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TECHNICAL MANUSCRIPT 580

EFFECT OF RECIPIENT CELL CONCENTRATION ON TRANSFECTION WITH BACTERIOPHAGE DNA

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BIOLOGICAL SCIENCES LABORATORIES

Project 1B061102B71A

January 1970

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ABSTRACT

Transfection of Bacillus subtilis 168 with low concentrations of SP-18 phage DNA (molecular weight = 1×10^8) was enhanced by using recipient cells at a concentration of 2×10^7/ml rather than the usual 2×10^8/ml. At low DNA concentrations, the slope of the dose-response curve was >1 at the high recipient cell concentration, but the slope was = 1 at the low recipient cell concentration. A similar effect was shown with SP-82 DNA, Ø1 DNA, and Ø25 DNA, all having molecular weights of approximately 1×10^8. However, Ø29 DNA (molecular weight = 2.5×10^7) gave a first-order dose-response curve at both high and low recipient cell concentrations. We interpreted our observations as Kelly hypothesized for the preservation of marker linkage in transformation; i.e., the lower concentration of recipient cells resulted in fewer DNA molecules being fragmented as a result of attaching to two cells. This interpretation of transfection enhancement is different from the previous explanation as an inhibition of an intracellular inactivation process.

CONTENTS

FIGURES

I. INTRODUCTION*

In our studies to determine why the infectivity of SP-18 phage DNA was nonlinear at low DNA concentrations, we observed that dilution of the recipient cells greatly increased the number of transfectants obtained with low concentrations of SP-18 DNA.

Our observation was similar to that reported by Kelly[1] in which the use of dilute suspensions of competent cells preserved the linkage of various markers in transformation. We felt it worthwhile to expand this observation and to study the use of diluted recipient cells for transfection by DNA of various bacteriophages.

II. MATERIALS AND METHODS

Phage SP-18 was isolated from soil by Dr. Ivan D. Goldberg in this laboratory. Phages Ø1, Ø25, and Ø29 were described by Kelly and Spizizen,[2] and phage SP-82 was described by Green.[3] All phage suspensions were assayed on phage assay agar (PA) seeded with 10^8 Bacillus subtilis 168 (ind⁻) spores as described by Thorne.[4] Cultures of B. subtilis 168 (ind⁻) were used to prepare SP-18, Ø1, Ø25, and Ø29 in TY broth[5] and SP-82 in H broth.[6]

Phage DNA was extracted by a modification of the phenol technique of Mandell and Hershey.[7] The DNA was dialyzed for 22 hours at 5 C against 0.15 M NaCl and 0.015 M citrate (SSC) at pH 8.0 to remove the phenol and was stored at 5 C. DNA was determined by the method of Burton.[8] All DNA preparations were tested for contamination with bacteria or viable phage by spreading samples on nutrient agar and by plating samples (after treatment with 50 µg of deoxyribonuclease) in PA agar seeded with 10^8 B. subtilis 168 (ind⁻) spores. Serial twofold dilutions of the DNA preparations were made in SSC.

Competent cells of B. subtilis 168 (ind⁻) were grown essentially by the procedure of Anagnostopoulos and Spizizen.[9] Transfections were carried out in 18- by 100-mm tubes containing 1 ml of recipient culture (approximately 2×10^8 or 2×10^7 cells) and 0.1 ml of DNA. The tubes were incubated for 45 minutes in a slanted position on a reciprocal shaker at 37 C. Pancreatic deoxyribonuclease (IX crystallized, Worthington) was added (50 µg in 0.05 ml) and incubation was continued for 5 minutes. Infectious centers were scored against 168 (ind⁻) by the soft agar overlay method in PA agar.

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III. RESULTS

SP-18 DNA showed a first-order relationship between DNA concentration and infectivity in the region from approximately 2 to 40 μg/ml, but at DNA concentrations below 2 μg/ml the infectivity was greater than first order (Fig. 1). To determine the relationship between infectivity of SP-18 DNA and cell concentration, we made serial twofold dilutions of the recipient cells in the presence of DNA at 10 μg/ml and 1.25 μg/ml (Fig. 2). At 1.25 μg DNA/ml, maximum transfection was demonstrated with 2×10^7 cells/ml; more or fewer cells resulted in fewer transfectants/ml.

The effect of SP-18 DNA concentration on infectious center formation at recipient cell concentrations of approximately 2×10^8 and 2×10^7 cells/ml is shown in Figure 3. The dose-response curve at the high cell concentration was the same as that in Figure 1, but at the low cell concentration, the dose-response curve remained first-order in the region from about 0.2 to 2 μg/ml. This resulted in a higher percentage transfection with a cell concentration of approximately 2×10^7 cells/ml; e.g., at a DNA concentration of 0.2 μg/ml, the transfection obtained with 2×10^7 cells/ml was more than 300% that obtained with 2×10^8 cells/ml.

The increase in percentage transfection obtained with diluted competent 168 (*ind*$^-$) cells could be explained if, in diluting the cells, we also diluted a competence inhibitor or extracellular nuclease. To test this possibility, competent 168 (*ind*$^-$) cells were washed to get rid of any extracellular competence inhibitor or nuclease. However, the dose-response curves from washed cells (Fig. 4) were the same as those obtained from nonwashed cells (Fig. 3). An alternative explanation for our observed increase in percentage transfection with diluted recipient cells was the hypothesis presented by Kelly[1] that a DNA molecule might adsorb to more than one bacterial cell at the same time, resulting in fragmentation of the molecule. Dilution of the competent cells would lower the chance of a DNA molecule adsorbing to more than one cell, lowering fragmentation, and thereby increasing transfection.

To determine if the use of diluted recipient cells affected transfection with other phage DNA, we tested phage SP-82, whose DNA has a molecular weight (1.3×10^8) similar to that of SP-18 DNA (1.0×10^8). SP-82 DNA does not give a first-order dose-response curve in any region of DNA concentrations unless an enhancement mechanism is used.[10] However, when a recipient cell concentration of approximately 2×10^7 was used, we obtained a first-order dose response with SP-82 DNA (Fig. 5). If large DNA molecules were fragmented by adsorption to more than one recipient cell, we would expect smaller DNA molecules to be less affected. To test this, we measured transfection with ϕ29 DNA, which has a molecular weight of 2.5×10^7. The ϕ29 DNA gave a first-order relationship between DNA concentration and infectious-center formation at the same DNA concentrations for both high and

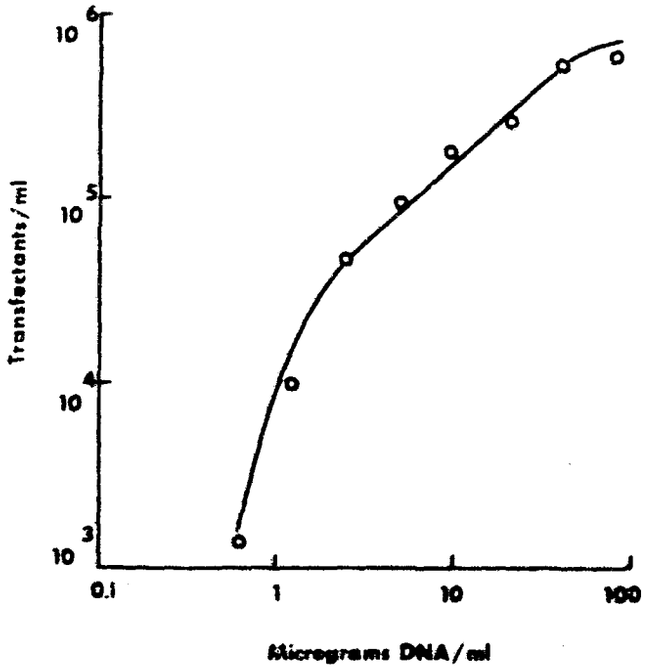

FIGURE 1. Effect of SP-18 DNA Concentration on Formation of Infectious Centers. ○ = approximately 2 x 10^8 recipient cells/ml.

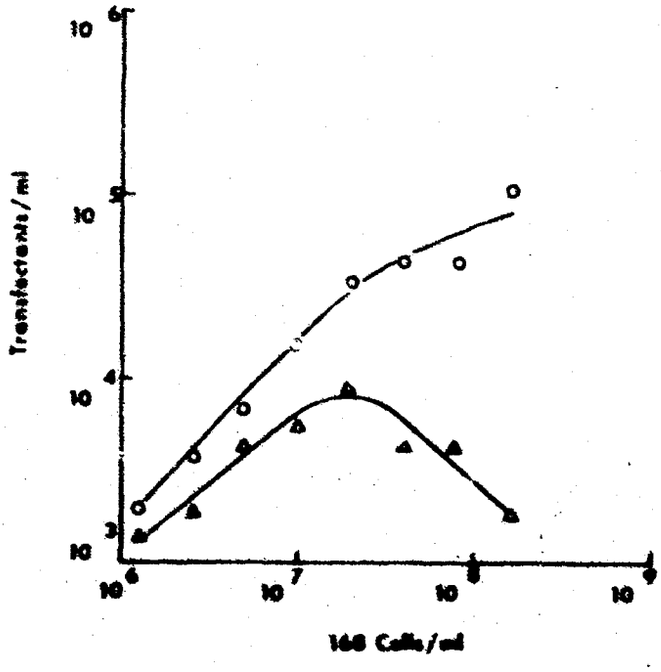

FIGURE 2. Effect of Recipient Cell Concentration on Formation of Infectious Centers. ○ = 10 μg SP-18 DNA/ml. △ = 1.25 μg SP-18 DNA/ml.

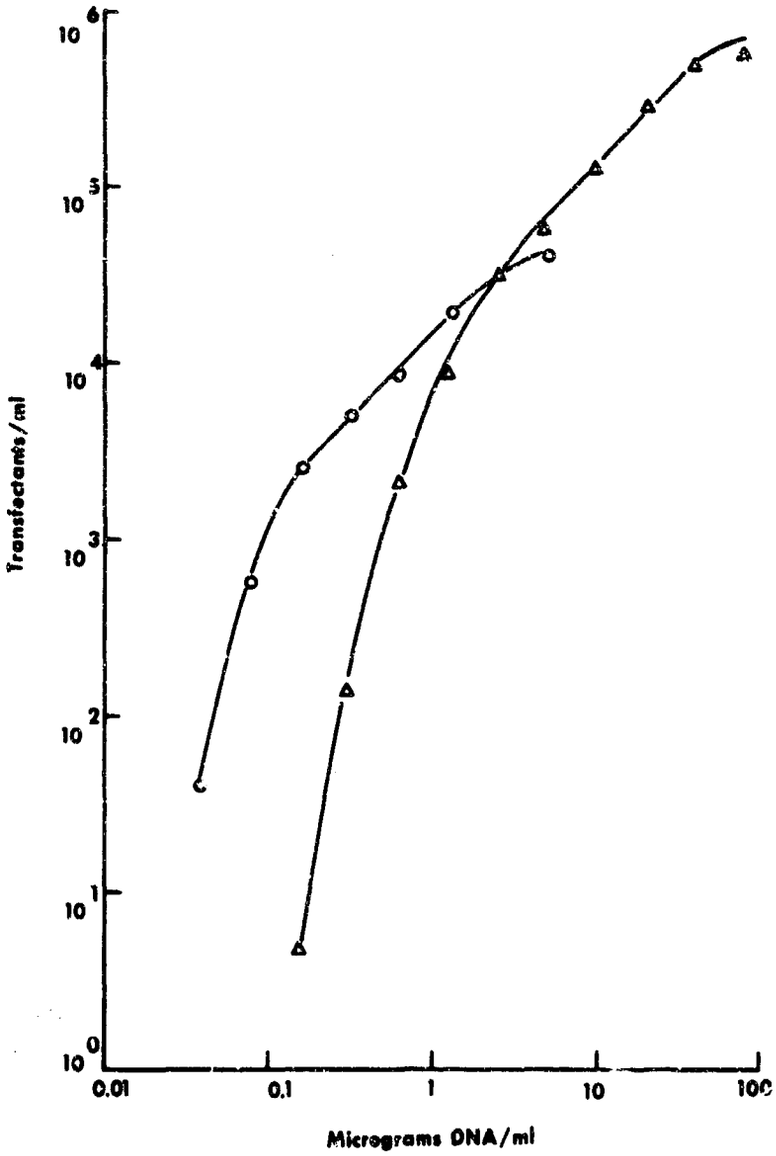

FIGURE 3. Effect of SP-18 DNA Concentration and Recipient Cell Concentration on Formation of Infectious Centers. ○ = approximately 2 x 10^7 recipient cells/ml, △ = approximately 2 x 10^8 recipient cells/ml.

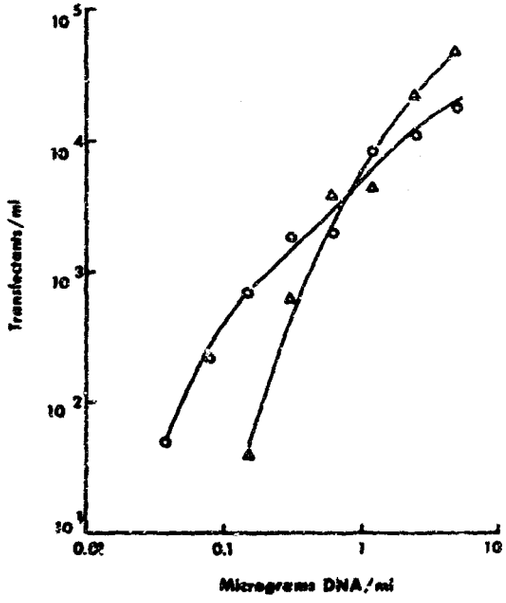

FIGURE 4. Effect of SP-18 DNA Concentration and Washed Recipient Cell Concentration on Formation of Infectious Centers. The competent 168 (ind⁻) cells were washed two times in competence media and resuspended in competence media to their original titer. ○ = approximately 2 x 10^7 recipient cells/ml, △ = approximately 2 x 10^8 recipient cells/ml.

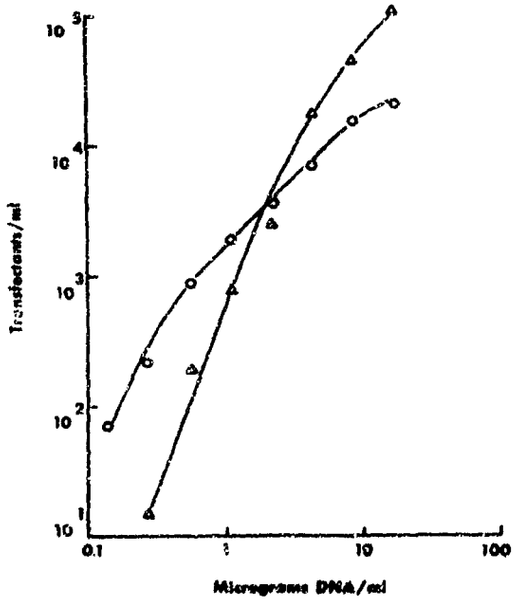

FIGURE 5. Effect of SP-82 DNA Concentration and Recipient Cell Concentration on Formation of Infectious Centers. ○ = approximately 2 x 10^7 recipient cells/ml, △ = approximately 2 x 10^8 recipient cells/ml.

low recipient cell concentration (Fig. 6). Our interpretation was that transfection with low molecular weight ϕ29 DNA was not affected by fragmentation of the DNA molecules resulting from adsorption to more than one recipient cell. Concentrations of ϕ29 DNA lower than those shown in Figure 6 gave a dose-response curve with a slope >1 at both high and low recipient cell concentrations.

To show that the first-order dose response obtained with low concentrations of recipient cells was not unique to SP-18 DNA and SP-82 DNA, we transfected high and low recipient cell concentrations with ϕ1 DNA and ϕ25 DNA, both having molecular weights of 1×10^8. The results (Fig. 7 and 8) supported our previous observations with high molecular weight DNA; we obtained a first-order dose-response curve with the low recipient cell concentrations but not with the high recipient cell concentration.

When phage DNA concentrations higher than those shown were added to high recipient cell concentrations, we observed a flattening of the dose-response curve or saturation of the system. It must be emphasized that a first-order dose response from SP-82, ϕ1, and ϕ25 DNA was obtained only with low recipient cell concentrations.

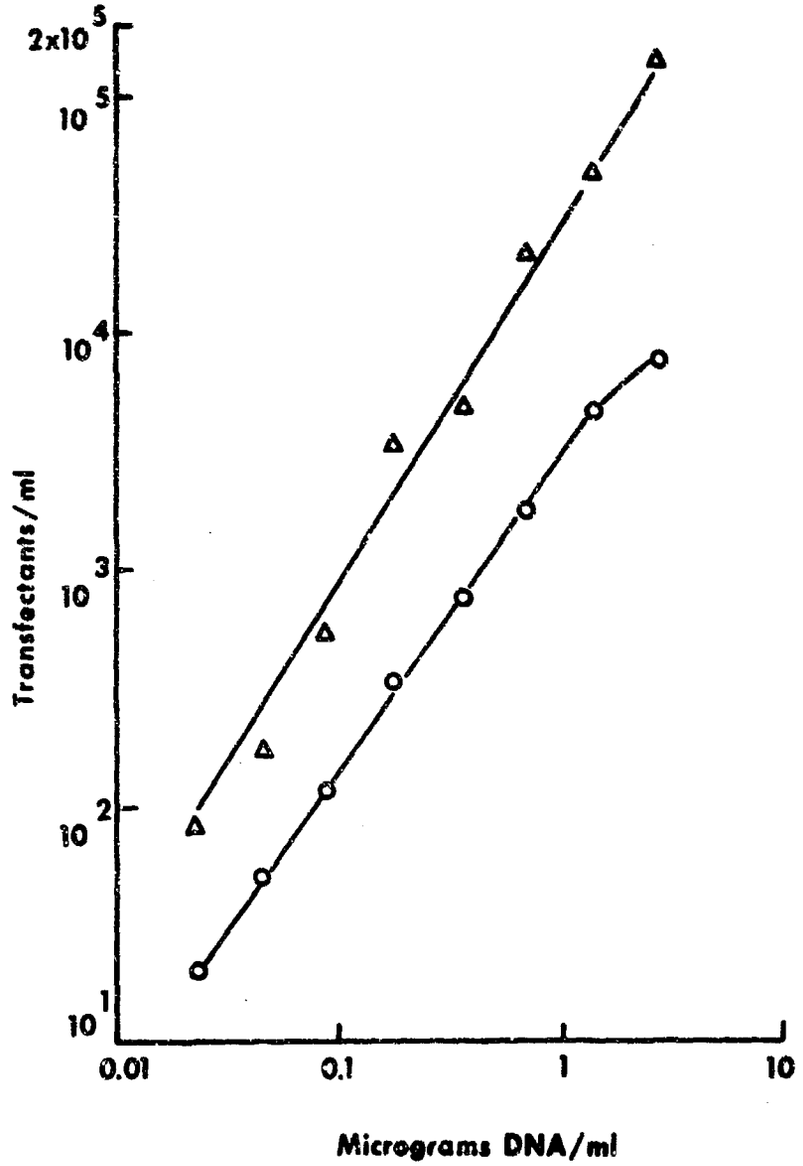

FIGURE 6. Effect of ϕ29 DNA Concentration and Recipient C-11 Concentration on Formation of Infectious Centers. ○ = approximately 2 x 10^7 recipient cells/ml, △ = approximately 2 x 10^8 recipient cells/ml.

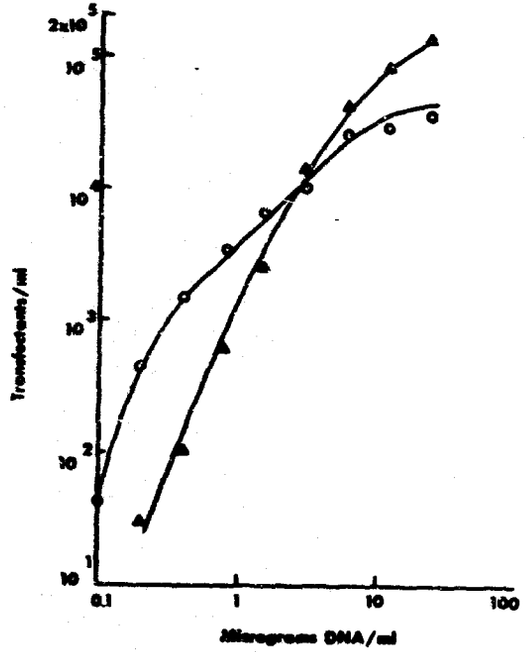

FIGURE 7. Effect of ϕ25 DNA Concentration and Recipient Cell Concentration on Formation of Infectious Centers. ○ = approximately 2 x 10^7 recipient cells/ml, Δ = approximately 2 x 10^8 recipient cells/ml.

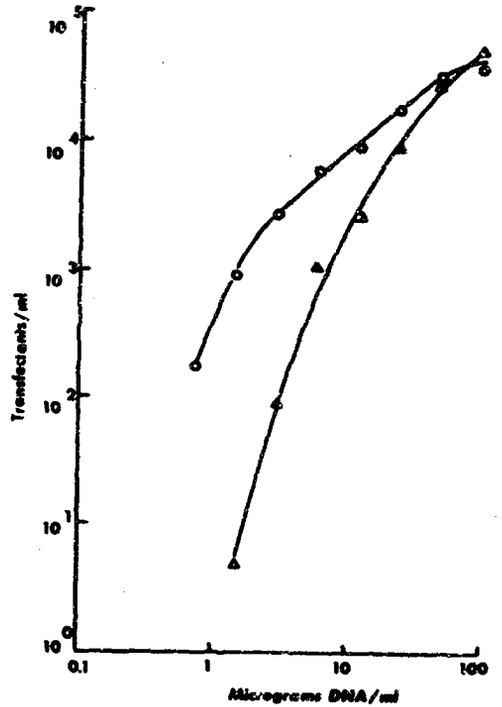

FIGURE 8. Effect of ϕ1 DNA Concentration and Recipient Cell Concentration on Formation of Infectious Centers. ○ = approximately 2 x 10^7 recipient cells/ml, Δ = approximately 2 x 10^8 recipient cells/ml.

IV. DISCUSSION

Our observations can be interpreted to support Kelly's hypothesis[1] that "at low concentrations of high molecular weight DNA, molecules normally attach to and are integrated into more than one recipient cell."

The dose-response curves from SP-82, Ø1, and Ø25 DNA using high recipient cell concentrations went from saturation to curves with slopes >1. There was no intermediate DNA concentration where a first-order dose response was obtained.

The first-order dose-response curves from SP-18 DNA, SP-82 DNA, Ø1 DNA, and Ø25 DNA that we observed at low concentrations of recipient cells change to greater than first order at the lowest DNA concentrations. The most probable explanation for this change is loss of contact with the recipient cells by the dilute, high molecular weight DNA, resulting in fewer infections being initiated.

SP-18 DNA gave a first-order dose response at both high and low recipient cell concentrations, but not at the same DNA concentration. SP-82 DNA, Ø1 DNA, and Ø25 DNA gave first-order dose responses only at low recipient cell concentrations. The genomes of these three phages may have weak points making them more susceptible to fragmentation.

SP-82 DNA, which normally shows a dose-response curve with a slope >1, has been reported to show a dose-response curve with a slope of one under certain conditions. Green[11] obtained a first-order curve with SP-82 by the use of helper phage, and he hypothesized that preinfection with mutant helper phage inhibited the intracellular inactivation process that he felt was responsible for the dose-response curve with a slope >1.

Epstein[12] enhanced SP-82 transfection by UV-irradiation of the recipient cells, and Epstein and Mahler[10] enhanced SP-82 transfection by pretransfection exposure of competent cells to UV-irradiated DNA.

LITERATURE CITED

1. Kelly, M.S. 1967. The causes of instability of linkage in transformation of Bacillus subtilis. Mol. Gen. Genet. 99:350-361.

2. Reilly, B.E.; Spizizen, J. 1965. Bacteriophage deoxyribonucleate infection of competent Bacillus subtilis. J. Bacteriol. 89:782-790.

3. Green, D.M. 1964. Infectivity of DNA isolated from Bacillus subtilis bacteriophage, SP-82. J. Mol. Biol. 10:438-451.

4. Thorne, C.B. 1962. Transduction in Bacillus subtilis. J. Bacteriol. 83:106-111.

5. Romig, W.R. 1962. Infection of Bacillus subtilis with phenol-extracted bacteriophages. Virology 16:452-459.

6. Chase, M.; Doermann, A.H. 1958. High negative interference over short segments of the genetic structure of bacteriophage T_4. Genetics 43:332-353.

7. Mandell, J.D.; Hershey, A.D. 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. 1:66-77.

8. Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of desoxyribonucleic acid. Biochem. J. 62:315-322.

9. Anagnostopoulos, C.; Spizizen, J. 1961. Requirements for transformation in Bacillus subtilis. J. Bacteriol. 81:741-746.

10. Epstein, H.T.; Mahler, I. 1968. Mechanisms of enhancement of SP-82 transfection. J. Virol. 2:710-715.

11. Green, D.M. 1966. Intracellular inactivation of infective SP-82 bacteriophage DNA. J. Mol. Biol. 22:1-14.

12. Epstein, H.T. 1967. Transfection enhancement by ultraviolet light. Biochem. Biophys. Res. Commun. 27:258-262.

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13. ABSTRACT

Transfection of Bacillus subtilis 168 with low concentrations of SP-18 phage DNA (molecular weight = 1 x 10^8) was enhanced by using recipient cells at a concentration of 2 x 10^7/ml rather than the usual 2 x 10^8/ml. At low DNA concentrations, the slope of the dose-response curve was >1 at the high recipient cell concentration, but the slope was = 1 at the low recipient cell concentration. A similar effect was shown with SP-82 DNA, Ø1 DNA, and Ø25 DNA, all having molecular weights of approximately 1 x 10^8. However, Ø29 DNA (molecular weight = 2.5 x 10^7) gave a first-order dose-response curve at both high and low recipient cell concentrations. We interpreted our observations as Kelly hypothesized for the preservation of marker linkage in transformation; i.e., the lower concentration of recipient cells resulted in fewer DNA molecules being fragmented as a result of attaching to two cells. This interpretation of transfection enhancement is different from the previous explanation as an inhibition of an intracellular inactivation process.

14. Key Words

Transfection
DNA
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Dose-response curve
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